Biological Soil Disinfestation with Organic Fermentation Products

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Abstract

Much research has been done on biological disinfestation of soil with grass and other fresh organic materials to suppress persistent diseases like *Verticillium dahliae* and nematode populations in the soil. After covering the soil with airtight plastic and creating an anaerobic environment, natural processes that will have a disinfesting effect on the soil are stimulated by the organic material. Practical conditions may vary enormously and so it seems that soil disinfestation with grass or other fresh materials is unpredictable. Growers find this method complicated and labor intensive. In particular, growers of horticultural crops are not enthusiastic about biological soil disinfestation with grass. As an alternative for fresh materials, organic fermentation products have been tested.

Application is easier and effects occur faster than with fresh organic materials. Therefore, this method offers a chance to explore the possibilities for broad application in horticulture as an alternative for soil disinfestation with steam. Product H7022 (ThatchTec B.V., Wageningen, The Netherlands), consists of organic by-products from the food processing industry, and resulted in a 100% reduction of nematodes and *Verticillium dahliae.* The major drawback to this method is the treatment time of at least two weeks, which is too long for most greenhouse growers, as the costs of a two week time gap in production are too high, making it difficult to adapt to a greenhouse system.

New research on organic fermentation of the soil is necessary to determine which processes cause soil disinfestation, during what time period this method causes an effect, and whether or not this method is suitable for use in soil-bound horticulture.

INTRODUCTION

Greenhouse horticulture has offered great opportunities for year-round cultivation of a broad range of crops in temperate climates. The temperature, watering and other growing factors can be easily adjusted to optimal ranges and the crop is protected against outside influences. Additionally, vegetable crops like tomato and cucumber are often grown in an artificial growing medium that is replaced after each production period, and the greenhouse is disinfested to ensure the next crop has a healthy start.

In contrast, growers of soil-bound crops, (e.g., organic vegetables and ornamental crops like Chrysanthemum), cannot make a new start by removing all organic material, soil and potential plant pathogens and pests. Continuous production of monocultures in greenhouses can lead to major problems and heavily infested soils (Runia et al., 2010). Soil-borne fungi like *Verticillium dahliae* and nematodes like *Pratylenchus penetrans* can lead to serious losses. These diseases and pests often have broad host ranges and force growers to disinfest their soils (Runia et al., 2010). In the past, soils were often fumigated with chemicals like methyl bromide which is toxic to humans and has a depleting effect on the ozone layer. In the Netherlands, this chemical has been delisted since January 1992 (Ministry HPPE, 1992). Since then, steam sterilization is commonly used as a method for soil disinfestation (Runia et al., 2010).

STUDIES

Steaming in Soil Bound Cultivations

In Dutch horticulture, steam sterilization is a method that is often used to disinfest the soil. Steaming is effective against soil fungi and nematodes if the soil comes to a temperature of 70°C for a period of at least half an hour (Bollen, 1969, 1985). There are two main methods of steaming. Sheet steaming is achieved by covering the soil with a large sheet, anchoring the edges and blowing large amounts of steam under it. This method is effective only on clay soils, may take up to eight hours to be completed and uses large amounts of gas (7 m^3 m^{-2} soil) (Runia, 2000). The alternative is negative pressure steaming which uses a drain system that is placed at 60 to 80 cm depth and is permanent and specially installed. This method consumes less energy (4 m^3 gas per m^2 of soil) and is applicable on all soil types (Runia, 2000). However, if a soil-heating system is laid out in the greenhouse at 30 cm depth, it is impossible to use this method of negative pressure steaming (Runia, 2000). Additionally, Soil disinfestation with steam does not always produce good results, has a relatively short-term effect, is unpopular with organic growers and consumes large amounts of fossil energy (Runia, 1982, 1984, 2000). Therefore, it is necessary to search for good soil disinfestation alternatives.

Alternatives for Steaming

In the search for an alternative to chemical fumigation and steaming, a method called biological soil disinfestation with grass and other fresh organic materials to suppress persistent diseases like *Verticillium dahliae* and nematode populations in the soil was developed (Blok et al., 2000). After covering the soil with airtight plastic and creating anaerobic conditions, natural processes that have a disinfesting effect on the soil are stimulated by the fresh material. In practice, conditions like soil type, temperature, pH, composition of the grass, etc. may vary enormously and so it seems that soil disinfestation with grass or other fresh materials is an unpredictable, although promising method. More information about the factors that influence the processes in the soil is needed, as the method is only applicable in horticulture if the results are effective, reliable and predictable.

As an alternative for grass, organic fermentation products have been tested. These organic fermentation products (ThatchTec B.V., Wageningen, The Netherlands) have a defined composition and can promote the natural processes in the soil which can shorten the time span needed for an effect and make the effect more predictable. Organic fermentation products are also easier to use than grass. These characteristics offer a chance to explore the possibilities for broad application in horticulture as an alternative for soil disinfestation with steam or chemicals. Lamers and Wilms (2008) found a long term effect of biological soil disinfestation with organic amendments. If this can also be demonstrated for organic fermentation products like H7022, it might have an influence on the practical application of this method.

Goal

The goal of this research project is to develop insight into the mechanisms of processes promoted by insertion of organic fermentation products into the soil under anaerobic conditions. Which elements influence the fermentation process and which processes influence the production of organic acids and gasses?

This project will answer the following questions:

1. What is the effect of several organic fermentation products on the survival of *Verticillium dahliae* and *Pratylenchus penetrans*?
2. Which conditions are crucial for the product's success?
3. Is there a correlation between the production of gasses and organic acids and survival of *V. dahliae* and *Pratylenchus penetrans*?

MATERIALS AND METHODS

The experiment was performed in 2009 in climate chambers at Applied Plant Research (PPO-AGV) in Lelystad, The Netherlands, with a constant temperature of 16°C and a relative humidity of 60–70%, within white airtight buckets (Superfos, Superlift 11.3 L, Ø 293 mm (No. 8116-6B) and accompanying lid No. 4116-1B) that were filled with 8 L of one of two different soil types. The experiment was performed in two replicates, with 3 treatment times (2, 4, 8 weeks) and with 3 dosages of H7022 (2, 4 and 6 g raw protein L^{-1} soil) and two soil types (light marine clay, high in organic matter (Ens, The Netherlands) and sand, low in organic matter (Vredepeel, The Netherlands). As controls, the same dosages of grass (2, 4 and 6 g raw protein L^{-1} soil), untreated soils in covered buckets (0 g of raw protein L^{-1} soil) and untreated soils in uncovered buckets (0 g of raw protein L^{-1} soil (natural mortality)) were used. Dosages were mixed homogenously over the amount of soil in the buckets and were brought to field capacity moisture level. Dry matter content of the sandy soil was 85.3% and of the light marine clay 74.2%. Inocula were added into the soil: *Pratylenchus penetrans* was added directly to the soil (500 nematodes 100 ml^{-1} soil), *Verticillium dahliae* was incorporated into the soil in bags (screen size of 50 µm) filled with 3 g of inoculum (made of ground up severely infected tomato stems). Bags with *V. dahliae* inoculum were buried at a depth of 10 cm in the soil of each bucket. The buckets were sealed during the experiment to create anaerobic conditions. Processes were monitored by sampling gasses in the airspace between the soil and the lid of the bucket. The buckets were equipped with a small metal tap. The tap was placed above soil level, a tube could be attached to the tap for direct measurements using a hand meter for oxygen (O_2) and hydrogen sulphide (H_2S) (Impact Pro, Zellweger analytics) and using an Innova 1412 photo acoustic Field Gas Monitor for greenhouse gas (carbon dioxide (CO_2), ammonia (NH_3), nitrous oxide (N_2O), methane (CH_4)) measurements. Gasses were monitored weekly during the experiment. The experiment lasted for 8 weeks. During this period, the bags filled with inoculum were collected after 2, 4 and 8 weeks. The inoculum was analyzed and quantified after treatments.

Pratylenchus penetrans was analyzed per 100 ml of soil by elutriation of the soil (Oostenbrink-elutriator). The content of the bag filled with *V. dahliae* inoculum microsclerotia were ground, and 0.003 g of the material was brought onto a specific *Verticillium* medium (Modified soil extract agar (MSEA) (Harris et al., 1993) in threefold. Germinating microsclerotia were scored. Results were analyzed using GenStat 12.1 (VSN International Ltd.). Analysis of variance was performed on the logarithm of the number of juveniles of *P. penetrans* and the number of microsclerotia of *V. dahliae*.

RESULTS

The product H7022 offered very good results at a constant soil temperature of 16°C, in comparison with a grass treatment and the untreated controls.

The results in the light marine clay showed that the number of juveniles of *P. penetrans* was reduced by more than 90% by both grass and the fermentation product H7022 at all three dosages (2, 4 and 6 g raw protein L^{-1} of soil) tested (Fig. 1).

Figure 2 shows that the organic fermentation product H7022 reduced the number of viable microsclerotia of *V. dahliae* to zero in the light marine clay after 8 weeks of anaerobic conditions. Grass did not eliminate all viable microsclerotia. Even higher dosages of grass (6 g raw protein L^{-1} soil) were not enough to eliminate all microsclerotia after 8 weeks.

In the sandy soil, the effects of both grass and the organic fermentation product H7022 were similar to those found in the clay soil on the reduction of numbers of *P. penetrans* (Fig. 3) and *V. dahliae* (Fig. 4). However, the number of viable microsclerotia of *V. dahliae* was reduced to zero 4 weeks earlier in treatments with H7022, compared with grass. The effect of grass and H7022 in comparison with a control treatment on *P. penetrans* and *V. dahliae* is visualized in Tables 1 and 2, respectively (Analysis of Variance ($P<0.05$)).

DISCUSSION

Based on the results of this experiment, we can conclude that application of H7022, in combination with an anaerobic environment achieved by covering the soil with an airtight plastic, can offer good results in reducing the amount of viable microsclerotia of *V. dahliae* and juveniles of root lesion nematodes (*P. penetrans*). Soil characteristics influenced the duration necessary for an effect to occur. This project also showed that the organic fermentation product H7022 had a significantly better or faster effect on the eradication of *V. dahliae* microsclerotia and *P. penetrans* juveniles in comparison with grass or with the untreated control.

The time necessary for complete elimination of microsclerotia of *V. dahliae* and *P. penetrans* juveniles, was dependent on soil characteristics and dosage of organic fermentation product H7022.

A remarkable phenomenon was the increase of viable microsclerotia of *V. dahliae* after 2 weeks of treatment (Figs. 2 and 4). This effect was visible for both soil types and in all treatments and dosages. This increase can possibly be explained by decomposition of the inoculum, which freed microsclerotia from the plant material. Another explanation could be that microsclerotia were stimulated because of the anaerobic environment, before they lost their viability.

The previously mentioned gas measurements during the process were indicative and offered some correlations between concentration and effect, but were not significant. These indications need to be confirmed in further research.

Although the results showed a good effect, the necessary period of production loss is too long for practical application from an economical point of view (Ludeking et al., 2010). However, it is expected that the exposure time that was necessary under controlled conditions will be shorter under practical conditions. Examples of practical applications of the method offer good results after 2–4 weeks' time under the influence of, for example, higher soil temperatures, sunlight and other factors in the field.

A duration of two weeks is acceptable for the intensive cultivation of chrysanthemum, if biological soil disinfestation is compared with soil steaming from an economical point of view (Ludeking et al., 2010).

More research is necessary to show which processes in the soil form the basis of soil disinfestation using organic fermentation products, to determine indicators of the process and find out how to speed up the method. Answering these questions may result in the development of a predictable, energy saving and optimal disinfestation method for soil bound (organic) horticulture, as an alternative to steam disinfestation.

ACKNOWLEDGEMENTS

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Tables

Table 1. Median of number of juveniles *P. penetrans* per treatment and time (weeks). Values in the same columns, without a common letter, are significantly different at a probability level of 0.05.

Treatment	Soil type					
	Light marine clay			Sand		
	Time (weeks)			Time (weeks)		
	2	4	8	2	4	8
Grass	5.5b	0.0a	1.1a	15.6b	11.2c	2.7a
H 7022 G	0.9a	0.0a	0.9a	0.0a	0.0a	0.0a
Sealed control	15.9b	12.9b	24.7b	125.3c	118.6d	125.2b

Table 2. Median of number of microsclerotia of *V. dahliae* per treatment and time (weeks). Values in the same columns, without a common letter, are significantly different at a probability level of 0.05.

Treatment	Soil type					
	Light marine clay			Sand		
	Time (weeks)			Time (weeks)		
	2	4	8	2	4	8
Grass	100.3a	44.1a	32.5b	111.9a	63.5b	3.9a
H 7022 G	89.1a	23.4a	1.3a	90.3a	1.5a	0.0a
Sealed control	74.1a	38.2a	45.0b	55.5a	43.2b	40.1c

Figures

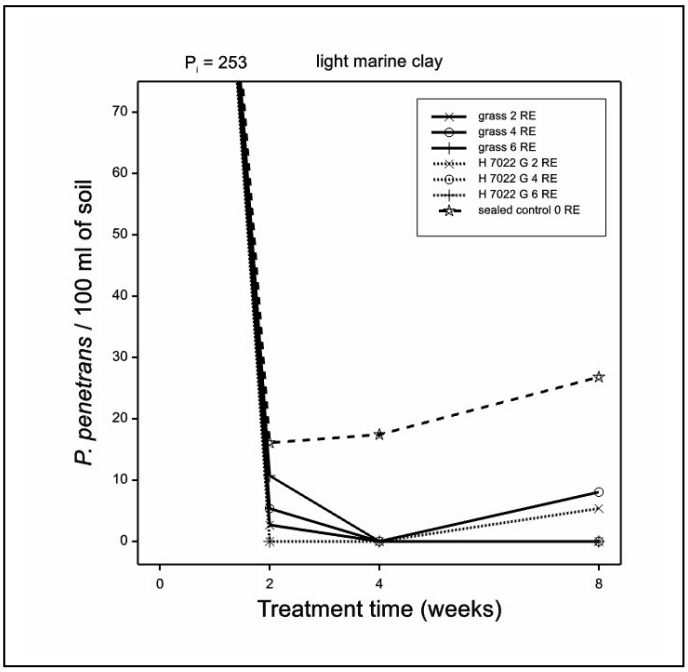

Fig. 1. Number of *Pratylenchus penetrans* juveniles per 100 ml light marine clay after treatment of 2, 4 and 8 weeks with 3 dosages (2, 4 and 6 g of Raw Protein (RE) L^{-1} soil) of grass or H7022.

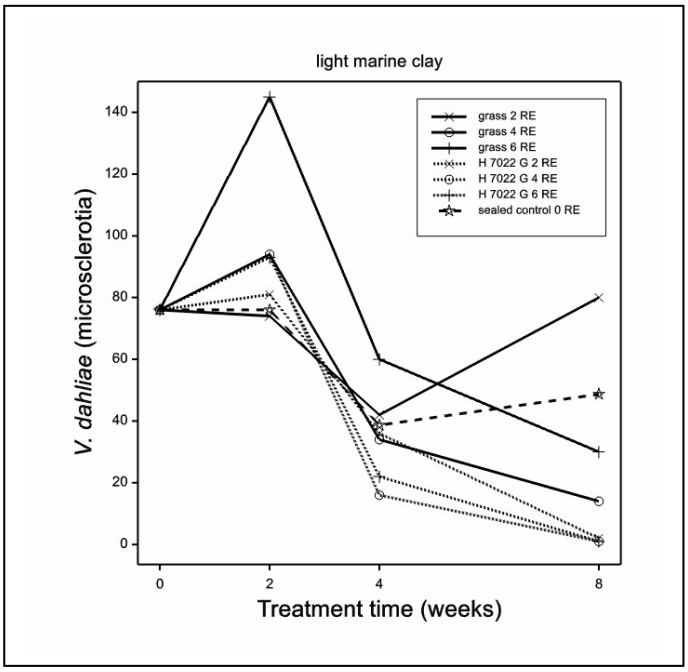

Fig. 2. Number of microsclerotia of *Verticillium dahliae* in light marine clay after treatment of 2, 4 and 8 weeks with 3 dosages (2, 4 and 6 g of Raw Protein (RE) L^{-1} soil) of grass or H7022.

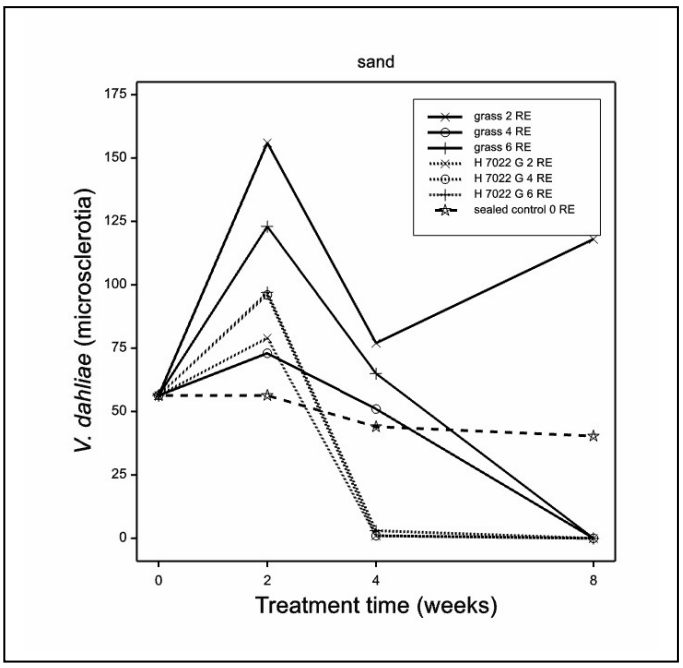

Fig. 3. Number of *Pratylenchus penetrans* juveniles per 100 ml sand after treatment of 2, 4 and 8 weeks with 3 dosages (2, 4 and 6 g of Raw Protein (RE) L^{-1} soil) of grass or H7022.

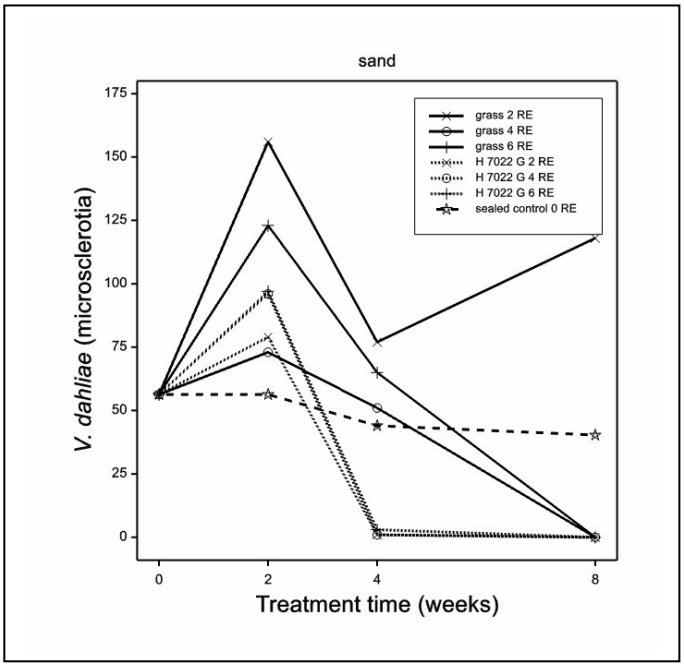

Fig. 4. Number of microsclerotia of *Verticillium dahliae* in sand after treatment of 2, 4 and 8 weeks with 3 dosages (2, 4 and 6 g of Raw Protein (RE) L^{-1} soil) of grass or H7022.